

EXHIBIT G
TO DECLARATION OF SCOTT D. TANNER, PHD.

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ICP-MS AS AN ELEMENTAL DETECTOR IN IMMUNOASSAYS. SPECIATION WITHOUT CHROMATOGRAPHY.

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We explore the application of ICP-MS as an elemental detector for elemental tags in immunoassays instead of the more common fluorescent tags. Metal-labeled physiologically (biologically) active materials can be used in specific binding assays as labeled antibodies, hormones, receptors, antigens, and so on. Tagging with a non-interfered tag (in the meaning of the elemental analysis) allows for interference-free determination, resulting in improved detectability. Further, specific tags for various species would allow simultaneous measurement of the various species. Finally, tagging with multiple isotopes allows for signal amplification proportional to the number of tags of the same tagging isotope. High specificity of the immunoassay allows the determination of the antigen in a sample without chromatographic pre-separation. We will demonstrate the feasibility of this analytical method by employing the tagging in the form of a gold nanoparticle, which is attached to an antibody without degrading its activity (tagged conjugate). After incubation of an antigen with the tagged conjugate, the sample is filtered through a size separating centrifugal filter. The non-reacted tagged antibody together with other components of the sample mixture including non-reacted antigen pass into the filtrate. Complexes of the antigen and antibody conjugate are left on the filter and after washing out can be stabilized in acidic solution. After purification, one need not worry about retaining the sample integrity during the following sample preparation, because the quantity of metal in solution will reflect the quantity of antigen in the original sample. Quantitative analysis is carried out using the ICP-MS technique. The optimal concentrations of all reagents for this system were determined in initial criss-cross serial dilution experiments. The practical concentration of reagent being quantitated was ensured to be within the dynamic range of the standard curve.